ONLINE METHODS

Mice. C57BL/6 and BALB/c mice were from Charles River Laboratory. OT-II, DO11.10, CD11c-DTR, 4get, *Il4*$^{-/-}$, caspase-1-deficient and control NOD/ShiLtJ mice were from The Jackson laboratory. *Tlr2*$^{-/-}$, *Tlr3*$^{-/-}$, *Tlr4*$^{-/-}$, *Tlr6*$^{-/-}$, *Tlr7*$^{-/-}$, *Tlr9*$^{-/-}$, *Myd88*$^{-/-}$, *Ticam1*$^{lps-2/lps-2}$ mice were from S. Akira. *Nalp3*$^{-/-}$, *Ipaf*$^{-/-}$ and *Asc*$^{-/-}$ mice were provided by V. Dixit. Langerin-EGFP-DTR mice were from K.A. Hogquist. All animal protocols were reviewed and approved by the Institute Animal Care and Use Committee of Emory University.

Reagents. Endotoxin-free OVA was from Profos. CpG-B and Ultrapure LPS (*Escherichia coli* serotype 0111:B4) were from Invivogen. Papain, diphtheria toxin, NAC, tempol, pertussis toxin and BW245C were from Sigma Aldrich. The RNA Mini kit for RNA isolation and RT-PCR kit were from Qiagen. The SuperScript First-Strand Synthesis System for cDNA generation was from Invitrogen. Recombinant mouse IL-12 was from PeproTech. Papain and OVA were labeled with Alexa Fluor 488 carboxylic acid, 2,3,5,6-tetrafluorphenyl ester (A30005) and Alexa Fluor 647 carboxylic acid or succinimidyl ester (A20006) according to the manufacturer's instructions. Tempol-containing poly(ketal) microparticles were synthesized as described[45] with slight modifications.

Antibodies. Purified anti–mouse CD16-CD32 (2.4G2), anti-CD28 (37.51), anti-CD3ε (145-2c11), anti-CD11c (N418), anti-CD4 (GK1.5), anti-CD8 (53-6.7), anti-CD45 (30-F11), anti-CD45RA (14.8), anti-B220 (RA3-6B2), anti-IA-E (2G9), anti-CD49b (DX5), anti-CD62L (MEL-14), anti-IgE (23G3), antibody to T cell antigen receptor α-chain variable region 2 (B20.1) or β-chain variable region 5 (MR9-4), anti-IL-4 (11B11) and anti-IFN-γ (XMG1.2) were from BD Pharmingen. Anti–mouse Thy-1.2 (53-2.1) was from eBioscience. Purified anti–mouse CD3ε (145-2c11) was from Biolegend. Phycoerythrin-conjugated antibody to mouse TSLP receptor (FAB5451P) and neutralizing antibody to mouse TSLP (152614) were from R&D Systems. Anti–mouse CD205 (NLDC145) was from Serotec. Anti-mouse EO6 was provided by J. Witztum. Fluorescein-conjugated F(ab′)$_2$ goat anti–mouse IgM, μ-chain specific (115-096-020), was from Jackson Immunoresearch. Antibody to mouse TSLP (L-18) was from Santa Cruz Biotechnology. Alexa Fluor 488–conjugated donkey anti–goat IgG (A11055) was from Invitrogen.

Immunization. C567BL/6 mice were immunized subcutaneously at the base of tail with 100 μg OVA plus 50 μg adjuvant (papain, bromelain or CpG) in 100 μl PBS. In some experiments, mice were boosted on days 7 and 14. Serum was collected on day 14 after the first immunization, and titers of IgG1, IgG2b or IgE anti-OVA were analyzed by ELISA. On day 21 after primary immunization, draining lymph node cells were isolated and then restimulated for 4 d *in vitro* with OVA. In some experiments, mice were immunized subcutaneously with 50 μg papain or bromelain only. For blockade of ROS by NAC, 4get mice were subcutaneously injected at the base of tail once daily with NAC (150 mg per kg body weight) from 2 d before to 3 d after immunization. For blockade of ROS in DCs *in vivo*, 6 mg poly(ketal) microparticles containing 300 μg tempol were injected into 4get mice subcutaneously at the base of the tail 24 h before immunization with OVA plus papain. In some experiments, 4get mice were injected subcutaneously with 0.5 μg pertussis toxin or 100 nM (100 μl) BW245c daily for 4 d at the site of immunization for inhibition of cell migration. For adoptive transfer of OT-II T cells, CD11c$^-$CD11b$^-$CD4$^+$ splenic T cells were purified by removal of CD11c$^+$ and CD11b$^+$ cells with anti-CD11c and anti-CD11b microbeads and the negative fraction was enriched for CD4$^+$ T cells with anti-CD4 microbeads. For analysis of proliferation, CD4$^+$ T cells were labeled with CFSE (carboxyfluorescein diacetate succinimidyl ester; Invitrogen).

Stimulation of lymphocytes. Draining lymph node cells were isolated by digestion for 20 min at 37 °C with collagenase type 4 (Worthington Chemicals). Samples were enriched for CD11c$^+$ DCs by positive selection with anti-CD11c magnetic beads (Miltenyi Biotech). For isolation of DC subsets, enriched CD11c$^+$ cells were stained with fluorescein isothiocyanate–conjugated anti-CD205 (NLDC-145), phycoerythrin-conjugated anti-CD45RA, peridinin chlorophyll protein–cyanine 5.5–conjugated anti-CD8α and allophycocyanin-conjugated anti-CD11c. Cells were sorted on a FACSAria. For isolation of basophils, draining lymph nodes were isolated 3 d after immunization of mice with papain plus OVA, then samples were enriched for basophils with anti-CD49b microbeads and then labeled with anti-CD49b and anti-IgE. CD49b$^+$IgE$^+$ cells were sorted by flow cytometry with a purity of >90%. Naive isolation of CD4$^+$ T cells first involved enrichment with anti-CD4 microbeads, followed by sorting of CD4$^+$CD62L$^+$ cells by flow cytometry. For *ex vivo* coculture of DCs, basophils and T cells, sorted CD11c$^+$B220$^-$ (conventional) DCs (5×10^3 to 10×10^3) or basophils (5×10^3 to 10×10^3) were cultured for 3–5 d with naive CD4$^+$ T (1×10^5) cells in 96-well round-bottomed plates in the presence of IL-3 (10 ng/ml; R&D Systems). Proliferation was assessed by [^{3}H]thymidine incorporation through the addition of ^{3}H-labeled thymidine (Amersham Life Sciences) to cells during the final 16 h of culture. For *in vitro* stimulation, purified splenic or lymph node DCs (1×10^4) were cultured with naive CD4$^+$ T cells (1×10^5) and OVA peptide (amino acids 329–339 (ISQVHAAHAEINEAGR); 10 μg/ml) in 200 μl complete RPMI medium (10% (vol/vol) FBS (vol/vol Cellgro), 2 mM L-glutamine (Gibco Invitrogen), 0.01 M HEPES (Lonza), pH 7.2, 1 mM sodium pyruvate (Lonza), 200 U/ml of penicillin-streptomycin (Lonza) and 0.055 mM β-mercaptoethanol (Gibco Invitrogen)) in 96-well round-bottomed polystyrene plates. In some experiments, papain (25 μg/ml), NAC (1.5 mM), anti-IL-12 (1 μg/ml; C18.2; eBioscience) or anti-CD70 (3 μg/ml; RF70; eBioscience) was added to cultures.

Isolation of dermal cells. Excised ear tissues were separated for exposure of the dorsal and ventral sides of the skin sheets and were allowed to float for 1 h at 37 °C with the dermal side down in a 0.25% (wt/vol) trypsin solution containing 2.5 mM EDTA. Epithelial sheets were peeled carefully from the dermis with forceps. Dermal sheets were minced into small pieces and were digested for 1.5–2 h at 37 °C with collagenase 4 (1 mg/ml) in complete RPMI-1640 medium for isolation of dermis-specific cells.

Immunohistology. Mice were killed at the appropriate time points and skin patches were isolated and snap-frozen in optimum cutting temperature compound (Triangle Biomedical Sciences), followed by immunofluorescence staining of sections 8–10 μm in thickness. For TSLP staining, air-dried sections were fixed with acetone for 10 min at −20 °C, followed by blocking for 30 min with antibody 2.4G2. Sections were blocked for 30 min at 25 °C with buffer containing 1% (wt/vol) BSA, 10% (vol/vol) donkey serum and 1% (wt/vol) 2.4G2, and were incubated for 2 h at 25 °C with goat anti–mouse TSLP (1:500 dilution; L-18; sc-19177; Santa Cruz Biotechnology). After samples were washed with PBS, Alexa Fluor 488–conjugated donkey anti–goat IgG (A11055; Invitrogen) was added for 1 h at 25 °C. For staining with EO6, sections were blocked with 10% (vol/vol) goat serum and were stained with EO6 (1 μg/ml). Fluorescein isothiocyanate–conjugated goat anti–mouse IgM (115-096-020; Jackson Immunoresearch was used as secondary antibody. All slides were mounted in Prolong antifade medium (Molecular Probes). Images were obtained with a Zeiss LCM510 confocal microscope. For staining of Langerhans cells, epidermal sheets were prepared and fixed in acetone for 10 min at −20 °C, followed by blocking for 30 min at 25 °C with 2.4G2 and incubation overnight at 4 °C with fluorescein isothiocyanate–conjugated anti–mouse IA-E.

Staining of ROS. For *in vitro* staining in DCs, total CD11c$^+$ lymph node DCs were stimulated *in vitro* overnight at 37 °C with papain (25 μg/ml). ROS were stained with the Image-iT LIVE Green Reactive Oxygen Species Detection kit according to the manufacturer's instructions (Invitrogen). For studies *in vivo*, C57BL/6 mice were injected subcutaneously with 50 μg papain, then cells from the draining lymph nodes were isolated 2 h later and incubated for 30 min at 37 °C with a solution of 25 μM DCF (5-(and-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate) in PBS. After being washed twice with PBS, cells were labeled with anti-CD11c and anti-CD45R (B220). For the identification of ROS in skin sections *in vivo*, mice were injected subcutaneously with 20 μg hydro-Cy5 at the site of immunization 1 h before tissue was collected. Skin patches were snap-frozen in optimum cutting temperature compound, then were cut into cryosections and labeled with Alexa Fluor 488–conjugated anti-CD11c. Images were captured by with a Zeiss LCM510 confocal microscope as described above.

Depletion of lineage-specific cells *in vivo*. For depletion of CD11c$^+$ cells *in vivo*, CD11c-DTR mice or littermate control mice received an intraperitoneal injection of diphtheria toxin (100 ng per mouse) 1 d before immunization[27]. For

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depletion of Langerhans cells, langerin-DTR mice received an intraperitoneal injection of diphtheria toxin (1 μg per mouse) 14 d before immunization. Ablation efficiency was monitored by analysis of DCs in lymph nodes, dermis or epidermal sheets. For depletion of basophils *in vivo*, mice were injected twice daily for 3 d with 5 μg anti-FcεRIα (MAR-1)[10]. The efficiency of basophil depletion was analyzed in peripheral blood 24 h after injection on day 3. For T cell depletion, mice were given daily intravenous injection of 40 μg anti-CD3 from 5 d before to 2 d after immunization.

ELISA. Titers of IgG1, IgG2a, IgG2b anti-OVA were assessed by ELISA as described[26]. IgE ELISA was done as described[58]. Cytokines in culture supernatants were detected with the OptEIA Set for sandwich ELISA from BD Biosciences.

Intracellular cytokine staining and analysis. $CD4^+$ T cells in culture for 4–5 d were restimulated for 5 h with anti-CD3 (10 μg/ml) and anti-CD28 (2 μg/ml) in 96-well flat plates (Nunc) in the presence of GolgiStop (BD Pharmingen). Cells were stained with fluorescein isothiocyanate–conjugated anti-CD4, were made permeable with Cytofix/Cytoperm (BD Biosciences) and were stained with phycoerythrin-conjugated anti-IL-4 (11B11; BD) and allophycocyanin-conjugated anti-IFN-γ before analysis on a FACSCalibur.

Microarray analysis. Total RNA was extracted from lymph node DCs stimulated *in vitro* with papain (25 μg/ml) or LPS (1 μg/ml) with an RNeasy kit (Qiagen). RNA quality was assessed with an Agilent Bioanalyser 2100, and only RNA with minimal degradation and distinct 18*S* and 28*S* rRNA bands was used for analysis. The Vanderbilt Microarray Facility did microarray processing. Fragmented and biotin-labeled cDNA was synthesized from 100 ng purified mRNA with the Ovation Biotin system (Nugen). The cDNA was hybridized to GeneChip MouseGenome 430 2.0 Array chips (Affymetrix). Hybridized chips were stained and washed and were scanned with a GeneArray scanner (Affymetrix). GeneSpring software (Silicon Genetics) was used for data analysis.

Statistics. The statistical significance of differences between groups was calculated with a two-tailed Student's *t*-test or one-way analysis of variance. *P* values of less than 0.05 were considered statistically significant.

58. McGowen, A.L., Hale, L.P., Shelburne, C.P., Abraham, S.N. & Staats, H.F. The mast cell activator compound 48/80 is safe and effective when used as an adjuvant for intradermal immunization with *Bacillus anthracis* protective antigen. *Vaccine* **27**, 3544–3552 (2009).